BJCP British Journal of Clinical Pharmacology

DOI:10.1111/j.1365-2125.2012.04222.x

Bioavailability and disposition of azelastine and fluticasone propionate when delivered by MP29-02, a novel aqueous nasal spray

Hartmut Derendorf,[1] Ullrich Munzel,[2] Ursula Petzold,[2] Joachim Maus,[2] Hermann Mascher,[3] Robert Hermann[4] & Jean Bousquet[5]

[1]Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville, FL 32610, USA, [2]MEDA Pharma GmbH & Co. KG, 61352 Bad Homburg, Germany, [3]pharm-analyt Labor GmbH, 2500 Baden/Vienna, Austria, [4]cr.appliance, Rossittenstrasse 15, 78315 Radolfzell, Germany and [5]Hopital Arnaud de Villeneuve University Hospital, Montpellier, and Inserm CSP1018, France

Correspondence

Dr Robert Hermann, cr.appliance, Rossittenstrasse 15, 78315 Radolfzell, Germany.
Tel.: +49 7732 820951
Fax: +49 7732 820953
E-mail: robert.hermann@cr-appliance.com

Keywords

azelastine hydrochloride, bioavailability, drug–drug interaction, fluticasone propionate, nasal spray, pharmacokinetics

Received

6 November 2011

Accepted

7 February 2012

Accepted Article Published Online

22 February 2012

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The topical second generation anti-histamine azelastine hydrochloride (AZE) and the potent corticosteroid fluticasone propionate (FP) are well established first-line treatments in allergic rhinitis (AR).
- MP29-02, a novel intranasal AZE and FP formulation, has been shown to control AR symptoms faster and better than standard intranasal AZE or FP.
- The systemic bioavailabilities of marketed AZE and FP nasal spray products have been established at about 40% and 1% only, respectively.
- For new combination medicinal products such as MP29-02, the determination of possible pharmacokinetic (PK) drug–drug interactions between both active components and formulation-based bioavailability alterations is essential.

WHAT THIS STUDY ADDS

- This paper provides for the first time information on potential drug–drug interactions, AZE and FP bioavailability and disposition characteristics of each component administered by the novel nasal spray formulation MP29-02.
- The studies employed highly sensitive FP and AZE LC-MS/MS assays and could therefore be conducted with recommended therapeutic doses, thereby circumventing previously recognized draw-backs that required nasal bioavailability studies to be conducted using supra-therapeutic doses.
- No significant PK drug–drug interaction between the active components AZE and FP was noted for MP29-02.
- AZE bioavailabilty was equivalent when MP29-02 data were compared with MP29-02-AZE-mono and Astelin®.
- Increased FP exposure was observed with MP29-02-based products compared with FP-BI. FP serum concentrations were generally very low with all investigational products suggesting no clinically meaningful pharmacodynamic differences in terms of systemic safety.

AIM(S)

To determine azelastine hydrochloride (AZE) and fluticasone propionate (FP) bioavailabilities of the novel nasal spray combination product MP 29-02, compared with MP29-02-based products containing only AZE (MP29-02-AZE-mono), FP (MP29-02-FP-mono), marketed AZE and FP single entity products (Astelin® and FP Boehringer-Ingelheim; FP-BI).

METHODS

Two randomized, three period, six sequence, three treatment crossover studies were conducted in healthy subjects. Study 1 administered 200 µg FP as MP29-02, MP29-02-FP-mono or FP-BI. Study 2 administered 548 µg AZE as MP29-02, MP29-02-AZE-mono or Astelin®. Each dose consisted of two sprays/nostril. Serum FP and plasma AZE were followed over 24 (FP) and 120 h (AZE) and quantified by LC-MS/MS. Peak (C_{max}) and total exposures AUC(0,t_{last}) were compared between the treatments by ANOVA.

RESULTS

Study 1: Average FP C_{max} was very low with all products (≤10 pg ml^{-1}). FP AUC(0,t_{last}) point estimates (90% CIs) for MP29-02 : MP29-02-FP-mono and MP29-02 : FP-BI ratios (%) were 93.6 (83.6, 104.7) and 161.1 (137.1, 189.3). Corresponding ratios for C_{max} were 91.0 (82.5, 100.4) and 157.4 (132.5, 187.1). Study 2: AZE AUC(0,t_{last}) point estimates (90% CIs) for MP29-02 : MP29-02-AZE-mono and MP29-02 : Astelin® ratios (%) were 98.8 (91.0, 107.4) and 105.5 (95.6, 116.4). Corresponding outcomes for C_{max} were 102.7 (92.1, 114.4) and 107.3 (92.6, 124.3).

CONCLUSIONS

No interactions of AZE and FP were found with the MP29-02 formulation. Azelastine bioavailability was similar for MP29-02 and Astelin®. Maximum and total FP exposure was higher for MP29-02-based products compared with FP-BI. FP concentrations were generally very low with all investigational products and did not suggest clinically meaningful differences concerning systemic safety.

Introduction

Allergic rhinitis (AR) is a global health problem increasing in prevalence, currently affecting more than 500 million people worldwide. Symptoms of AR affect social life, sleep, learning and working, thereby translating into a substantial burden [1].

Guidelines recommend H_1-antihistamines as first line therapy for AR [2, 3] while intranasal corticosteroids (ICS) are the gold standard treatment in patients with more severe symptoms, particularly nasal congestion [2–4]. However, surveys of practice patterns show that over 60% of AR patients were dissatisfied with their current treatment due to insufficient efficacy, indicating a still existent and significant unmet medical need [5].

MP 29-02, a novel azelastine hydrochloride (AZE) and fluticasone propionate (FP) containing product using an optimized intra-nasal formulation, was found to control AR symptoms more effectively and faster in patients with moderate and severe AR than intranasal AZE or FP alone [6–10].

In the clinical development of new combination medicinal products, possible drug–drug interactions between active components (either occurring in the formulation in terms of a pharmaceutical interaction or resulting in altered pharmacokinetics *in vivo*) or formulation-based bioavailability alterations need to be addressed [11].

This paper presents the results of two separate pharmacokinetic studies, which together addressed two major mechanistic objectives, i.e. (i) exclusion or characterization of a potential pharmacokinetic interaction between the two active components in the novel product MP29-02 and (ii) exclusion or characterization of potential formulation-based product differences in the nasal bioavailability of FP and AZE as compared with the marketed single entity products. Besides these mechanistic objectives the two studies served the overall regulatory objective to compare the systemic exposure data of MP29-02 with the already marketed AZE and FP mono products in order to confirm its systemic safety.

Study 1 compared MP29-02, a novel 0.1% AZE and 0.0365% FP nasal spray product with two different FP single entity products, namely a MP29-02-based single entity FP product without AZE (MP29-02-FP-mono) and a marketed comparator product (FP-BI, FP Nasal Spray, Boehringer Ingelheim/Roxane Laboratories, Columbus, OH, USA) and examined the relative bioavailability and disposition of FP. For the investigation of a potential alteration of the FP bioavailability/pharmacokinetics by the second active component AZE the novel product MP29-02 was compared with the MP29-02-FP-mono product. For the investigation of a potential formulation effect on the FP bioavailability MP29-02 was compared with the marketed comparator product FP-BI.

Study 2 followed the same conceptual approach by comparing the novel MP29-02 product with two different AZE-single entity products, namely a MP29-02-based single entity AZE product without FP (MP29-02-AZE-mono) and a marketed AZE comparator product (Astelin®; Meda Pharmaceuticals, Somerset, New Jersey, USA). For the investigation of a potential alteration of the AZE bioavailability/pharmacokinetics by the second active component FP the novel product MP29-02 was compared with the MP29-02-AZE-mono product. For the investigation of a potential formulation effect on the AZE bioavailability MP29-02 was compared with the marketed comparator product Astelin®.

The investigational MP29-02-based single entity products (i.e. MP29-02-FP-mono and MP29-02-AZE-mono) were exclusively developed for the mechanistic purposes of the present studies and are not intended for further clinical development.

Local and systemic adverse effects were assessed as well.

Methods

Setting

Both studies were conducted at the same clinical site (ClinPharmCologne, Cologne, Germany).

Participants

Male and female participants were required to be healthy, non-smokers, aged 18–45 years and to have no history or evidence of any clinically allergic, respiratory or other disease. Subjects were excluded if they had a history of allergic reactions or sensitivity to FP, AZE or any of the excipients, a BMI >30 kg m^{-2}, QT_c interval >450 ms, abnormal routine blood tests, were taking any concomitant medications or were not able to demonstrate the correct self-application with an isotonic sodium chloride containing placebo nasal spray.

All subjects gave written informed consent, after approval by the ethics committee of Nordrhein Medical Council (*Ärztekammer Nordrhein*).

Design of the studies

A single centre, randomized, open label, three period, six sequence, three treatment crossover, pharmacokinetic single dose design was employed for both studies. At three separate occasions all subjects self-administered two sprays per nostril of the investigational products (200 µg FP and/or 548 µg AZE) in randomized sequence under the supervision of a clinical team member. Each study participant was thoroughly trained at screening and at each study period in the evening before the treatment days in self application of the nasal spray products, including proper operation of the nasal spray head. Each application was monitored for any detectable deviations from practiced procedure and observed deviations were detailed and documented in the case report form. Study periods were separated by wash-out intervals of at least 10 days.

Study 1: Effects of azelastine hydrochloride (AZE) or MP29-02 formulation effects on the relative bioavailability of fluticasone propionate (FP)

This study compared MP29-02, a novel 0.1% AZE and 0.0365% FP nasal spray product with two different FP single entity products. A MP29-02-based single entity FP formulation without AZE (MP29-02-FP-mono) and a marketed comparator product (FP-BI, FP Nasal Spray, Boehringer Ingelheim/Roxane Laboratories) (Table S1 'Composition of the study medication') served as reference products.

Serial blood samples for pharmacokinetic analysis of FP in serum were collected pre-dose and 8, 15, 30, 45 min, 1, 1.25, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 h after administration of study medication in each of the treatment periods. Although the mean terminal disposition half-life of FP after intravenous administration is reported to be 7.8 h, the duration of the employed FP sampling schedule of 24 h was considered adequate for the characterization of nasal spray formulations, because of the expected very low systemic exposure and after consideration of published evidence that FP serum/plasma concentrations upon intranasal FP administration could be hardly detected beyond 4 h post dose [12–14]. Hence, the 24 h sampling already took the significantly improved sensitivity of the employed LC-MS/MS assay with a lower limit of quantification (LLOQ) of 0.25 pg ml^{-1} into account. The zero (0 min) timepoint for post dose PK sampling was defined as the time the second spray into the second nostril was completed.

Study 2: Effects of fluticasone propionate (FP) or MP29-02 formulation effects on the relative bioavailability of azelastine hydrochloride (AZE)

This study compared MP29-02 with two different AZE single entity products, i.e. a MP29-02-based formulation without FP (MP29-02-AZE-mono) and a marketed comparator (Astelin®; Meda Pharmaceuticals, Somerset, NJ, USA) (Table S1 'Composition of the study medication').

Serial blood samples for pharmacokinetic analysis of AZE in plasma were taken pre-dose and 15, 30 min, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 48, 72, 96 and 120 h after administration of study medication. The AZE PK sampling schedule was based on available evidence that the mean terminal plasma disposition half-life of AZE is about 20 h [15].

Analytical methods

Study 1 (FP) Serum concentrations were quantified by a specific and highly sensitive liquid chromatography with tandem mass spectrometry (LC-MS/MS) assay with a lower limit of quantification (LLOQ) of 0.25 pg ml^{-1} and a validated FP concentration range up to 50 pg ml^{-1}. Inter-assay coefficients of variation of the calibration samples ranged between 1.69 and 7.87% and accuracy ranged from 98.9 to 101.2% (further details are provided in Items S1 and S2).

Study 2 (AZE) Plasma concentrations were quantified by a specific and highly sensitive LC-MS/MS assay with a LLOQ of 2.0 pg ml^{-1} and a validated AZE concentration range up to 1000 pg ml^{-1}. Inter-assay coefficients of variation of the calibration samples ranged between 1.60 to 4.96% and accuracy ranged from 93.2 to 103.2% (further details are provided in Items S3 and S4).

For both studies, the analyses from all periods of a subject were done in a single analytical run.

Pharmacokinetic analyses

Drug concentration–time data for each subject were analyzed by standard non-compartmental pharmacokinetic methods. PK parameters were calculated using a validated Excel based software (FUNCALC 3, 2003) (Item S5). For the purpose of product comparison of FP and AZE exposure characteristics, C_{max} and AUC(0,t_{last}) were considered the primary PK outcome variables for the assessment of rate and extent of absorption.

The analysis of all PK variables was performed on the per protocol (PP) population, which comprised all randomized subjects without major protocol deviations with potential relevance for the PK analyses.

Sample size considerations and statistical analyses

The studies were primarily designed and powered to assess the drug interaction potential between the active components since fixed combinations of AZE and FP have not been developed before and to meet respective regulatory requirements for fixed-combinational products [11].

The study protocol and the sample size planning, however, was not based on any mechanistic hypotheses on specific sources/mechanisms of potential exposure differences. It rather defined which extent of exposure differences between products would be acceptable in terms of systemic product safety. Hence, the statistical sample size consideration did not aim for the demonstration of bioequivalence. It rather referred to boundaries for systemic exposure differences (acceptance margins) that were deemed to be acceptable from a clinical safety perspective, thereby taking the expected very low systemic exposure levels upon intranasal administration and the known PK–PD relationship for the component with the lowest systemic safety margin (i.e. FP) into account (for further details see discussion section of the manuscript).

Based on these considerations an acceptance range of 50–200% for the Test : Reference ratios of the primary PK parameters was considered appropriate and pre-specified in the study protocols. The power calculation aimed for a sufficient sample size to achieve 80% power for the comparison of the AUCs of MP29-02 with MP29-02-FP-mono and MP29-02-AZE-mono, respectively, thereby assuming a true treatment ratio of 130% and a coefficient of variation (CV) of 60%. For this scenario a sample size of 24 subjects was calculated for each study to achieve 80% power. To account for potential drop-outs and for robustness in case of deviations from these assumptions a total of 30 subjects were randomized into each study.

Metrics indicating peak exposure (C_{max}), total exposure (AUC(0,t_{last}), AUC(0,∞)) and elimination (CL/F) were analyzed by using a crossover analysis of variance (ANOVA) after logarithmic transformation. The model included fixed effects treatment, period and sequence. The covariance structure over the treatments was unspecified. A Satterthwaite approximation was applied to the degrees of freedom. This model was used to provide geometric mean point estimates and 90% confidence intervals (CIs) for ratios MP29-02 : MP29-02-FP-mono and MP29-02 : FP-BI (Study 1) or MP29-02 : MP29-02-AZE-mono or MP29-02 : Astelin® (Study 2), respectively.

Safety and tolerability assessments

Safety evaluations included the assessment and documentation of the subjects' well being throughout the study in terms of nature, severity and incidence of adverse events. Medical history, ECG recordings, haematology, blood chemistry and urinalysis variables were assessed and a full physical examination performed as criteria for study enrolment. Vital signs were monitored at the beginning and end of the study.

Results

Subject demographics and disposition

Baseline demographic data of the study populations are given in Table 1. Both study populations had similar demographic characteristics.

Study 1 (FP) Thirty subjects were randomized and exposed to at least one dose of study medication. Two subjects discontinued the study prematurely, one subject because of a positive drug screening and another withdrew consent. Twenty-eight subjects completed all three study periods. Nine of them did not qualify for the PP analysis due to observed self-administration issues on at least one occasion (two for MP29-02, three for MP29-02-FP-mono, four for FP-BI). Because the dosing technique could affect PK results, PK analysis used the PP population of 19 subjects (for details on subjects' disposition see Table S2) and safety data included 30 subjects. A sensitivity analysis that included patients with documented administration issues yielded results consistent with the PP population and is included in Item S6.

Study 2 (AZE) Thirty subjects were randomized and all subjects completed the three study periods. Four subjects were excluded from the PP analysis because of protocol deviations with possible relevance for the PK analyses. Two had noted administration issues and two had local nasal findings with possible impact on local absorption (for details on subjects' disposition see Table S2). PK results refer to a PP population of 26 subjects and safety data to 30 subjects.

Safety and tolerability

All investigational treatments were well tolerated (for details on reported adverse events see Item S7).

Pharmacokinetics

Mean concentration–time profiles of FP and AZE for all five investigational nasal sprays are displayed in Figures 1 and 2. Geometric and arithmetic mean PK parameters (median for t_{max}), ranges, SDs and ANOVA derived coefficients of variations (CV%) are presented in Tables 2 and 3 for FP and AZE, respectively. Table 4 lists the mean point estimates (treatment ratio of MP29-02 : single entity product parameter) along with corresponding 90% confidence intervals for FP and Table 5 lists the same values for AZE.

Table 1

Subjects demographic characteristics at baseline (randomized safety population, n = 30)

Randomized safety population		Study 1 (FP) (n = 30)	Study 2 (AZE) (n = 30)
Gender	Male : Female (n)	18:12	18:12
	Male : Female (%)	60:40	60:40
Age (years)	Mean ± SD	30.2 ± 8.0	31.0 ± 6.8
	Median (range)	28.5 (18–45)	31.5 (19–44)
Race	Caucasian : Black : Indian : Mixed	27:2:1:0	28:0:0:2
Weight {kg)	Mean ± SD	73.3 ± 11.4	77.1 ± 12.6
	Median (range)	72.9 (53.9–104.2)	76.9 (56.1–107.3)
BMI (kg m^{-2})	Mean ± SD	24.1 ± 2.8	24.2 ± 2.6
	Median (range)	24.3 (19.2–29.9)	23.9 (19.2–29.1)

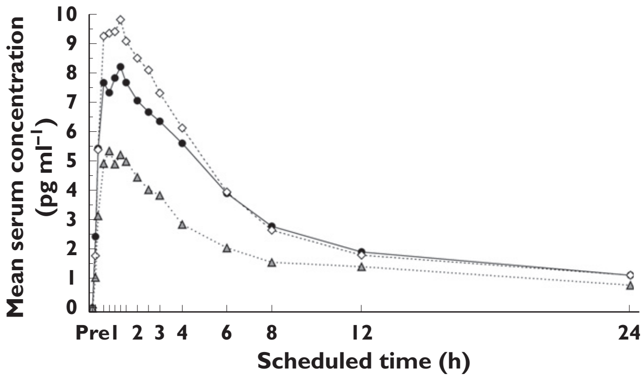

Figure 1

Study 1: Mean fluticasone propionate (FP) serum concentration–time curves after intranasal single dose administration of 200 µg FP delivered by three different nasal spray products; ● MP29-02; ◇ MP29-02-FP-mono (i.e. MP29-02 formulation without azelastine); △ Marketed comparator product FP-BI (Fluticasone propionate Boehringer-Ingelheim/Roxane Laboratories Nasal Spray)

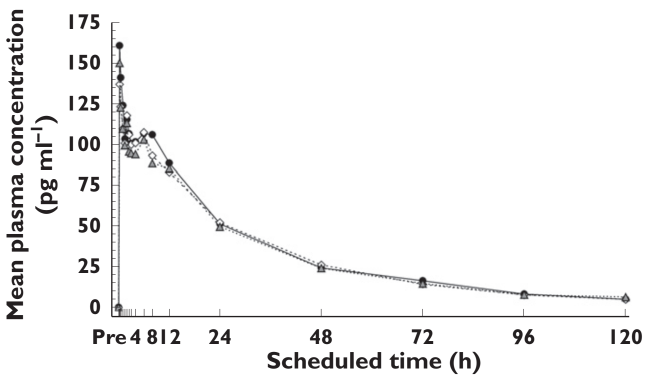

Figure 2

Study 2: Mean azelastine (AZE) plasma concentration–time curves after intranasal single dose administration of 548 µg AZE delivered by three different nasal spray products; ● MP29-02; ◇ MP29-02-AZE-mono (i.e. MP29-02 formulation without FP); △ Marketed comparator product (Astelin® Nasal Spray, Meda Pharmaceuticals)

Study 1 (FP) FP concentrations could be quantified throughout the 24 h post dose sampling with one exception for one subject (C_{last} at 12 h with FP-BI). Pre dose serum concentrations were below LLOQ in all periods in all subjects, which is consistent with an adequate wash-out interval between study treatments. All treatments resulted in rapid absorption of FP from the nasal mucosa with median t_{max} values within 1.0 h after dosing. Serum FP profiles were similar after MP29-02 and MP29-02-FP-mono. Respective geometric mean C_{max} values were 9.6 and 10.5 pg ml^{-1} and corresponding mean AUC(0,t_{last}) values were 61.9 and 65.7 pg ml^{-1} h. Peak and total FP systemic exposure from marketed FP-BI was somewhat lower with geometric means for C_{max} of 6.1 pg ml^{-1} and AUC(0,t_{last}) of 37.9 pg ml^{-1} h. Despite this numerical difference, maximum FP exposure was generally low, indicating an overall very limited systemic FP bioavailability for all investigational treatments. Mean FP serum concentration–time profiles displayed a rapid initial increase that peaked around 45 min post dose, with a discrete transient second peak at about 1 h 15 min post dose consistently noted with all products.

The concentration–time profile of the marketed comparator treatment (FP-BI) could be distinguished from the MP29-02-based treatments, with consistently lower concentrations at all time points over the first 8 h post dose. From 12 h post dose onwards, however, no meaningful differences between any of the treatments were notable. Average concentration–time curves displayed an apparent tri-exponential decline of FP serum concentrations with a sustained decline of very low residual concentrations (in most cases in the range of about 3.0 to 0.5 pg ml^{-1}) from 12 h post dose onwards. This long terminal disposition phase of FP has not been captured and described in previous studies using less sensitive assays, and represents therefore an unexpected finding. Consequently, in many subjects, the extrapolated fraction of the AUC(0,∞) values exceeded the cut-off value of 20% of the total AUC. Therefore, AUC(0,t_{last}) was selected to estimate total drug exposure. Table 4 shows C_{max} and AUC(0,t_{last}) ratios (and corresponding 90% CIs) of MP29-02 : MP29-02-FP-mono and indicate equivalent exposure. The corresponding ratios of MP29-02 : FP-BI point to a difference of about 60% for maximum and total average FP exposure.

Study 2 (AZE) The mean concentration–time profiles of all treatments were essentially identical and are nearly superimposable as shown in Figure 2. As with FP, AZE pre-dose plasma concentrations were below LLOQ for all periods in all subjects and are consistent with adequate duration of wash-out intervals. The rate of absorption was very rapid with initial peak concentrations at 15 min post dose followed by a transient decline. Median t_{max} values were consistently noted for all treatments at or in less than 0.5 h. Somewhat lower secondary concentration peaks were seen at 2 h post-dose followed by a further transient decline in AZE plasma concentrations. Finally, third peaks were observed at 6 h post dose. The results of the ANOVA analysis actually confirm equivalent systemic maximum and total AZE exposure in terms of C_{max} and AUC(0,t_{last}) for the comparison of MP29-02 with both AZE single entity products.

Discussion

These PK studies were part of the clinical development programme of MP29-02, a novel AZE and FP containing nasal spray product. Neither study showed any evidence of pharmacokinetic-based drug–drug interactions between the active components.

The very low systemic FP bioavailability (about 1%) from the nasal FP spray was attributed to poor aqueous

Table 2

Study 1: Summary of fluticasone propionate (FP) PK characteristics (PP population, $n = 19$)

Parameter (PP)	MP29-02	MP29-02-FP-mono*	FP BI†
AUC(0,t_{last}) (pg ml^{-1} h)	(n = 19)	(n = 19)	(n = 19)
Geometric mean (range)	61.9 (26.2–144.7)	65.7 (27.9–187.2)	37.9 (20.8–89.2)
CV (%)	52.1	54.6	47.0
Arithmetic mean ± SD	70.1 ± 36.5	74.0 ± 40.4	41.5 ± 19.5
t_{max} (h)	(n = 19)	(n = 19)	(n = 19)
Median (range)	1.00 (0.25–2.50)	0.75 (0.50–2.50)	1.00 (0.50–3.00)
Mean ± SD	0.98 ± 0.50	0.99 ± 0.49	1.13 ± 0.59
C_{max} (pg ml^{-1})	(n = 19)	(n = 19)	(n = 19)
Geometric mean (range)	9.6 (5.3–18.2)	10.5 (5.7–27.4)	6.1 (3.0–16.0)
CV (%)	37.7	50.2	48.6
Arithmetic mean ± SD	10.3 ± 3.88	11.7 ± 5.85	6.7 ± 3.23
$t_{1/2}$ (h)	(n = 16)	(n = 19)	(n = 18)
Median (range)	10.0 (6.2–30.8)	13.0 (8.6–26.8)	14.0 (7.0–100.4)
Mean ± SD	13.6 ± 7.5	14.6 ± 5.6	20.6 ± 21.9
CL/F (l h^{-1})	(n = 16)	(n = 19)	(n = 18)
Geometric mean (range)	2265.0 (1124.0–5673.2)	2278.4 (922.1–4247.7)	3380.5 (816.3–7059.3)
CV (%)	51.8	43.7	50.8
Arithmetic mean ± SD	2534.7 ± 1313.3	2512.3 ± 1097.3	3935.4 ± 1997.4

*MP29-02 formulation without AZE; †Boehringer-Ingelheim/Roxane Laboratories.

Table 3

Study 2: Summary of azelastine (AZE) PK characteristics (PP population, $n = 26$)

Parameter (PP)	MP29-02	MP29-02-AZE-mono*	Astelin®†
AUC(0,t_{last}) (pg ml^{-1} h)	(n = 26)	(n = 26)	(n = 26)
Geometric mean (range)	3 487.0 (1 466.7–11 430.7)	3 476.4 (1 608.2–9 163.9)	3 270.9 (1 299.3–8 647.1)
CV (%)	56.7	46.5	53.7
Arithmetic mean ± SD	3 949.2 ± 2 238.0	3 821.2 ± 1 774.7	3 696.6 ± 1 986.4
t_{max} (h)	(n = 26)	(n = 26)	(n = 26)
Median (range)	0.50 (0.25–12.0)	0.50 (0.25–12.0)	0.38 (0.25–8.0)
Mean ± SD	2.30 ± 3.42	1.65 ± 2.70	1.74 ± 2.66
C_{max} (pg ml^{-1})	(n = 26)	(n = 26)	(n = 26)
Geometric mean (range)	180.9 (85.7–363.5)	169.7 (68.1–307.2)	164.6 (52.1–388.4)
CV (%)	38.3	33.2	40.7
Arithmetic mean ± SD	194.5 ± 74.4	179.7 ± 59.6	178.3 ± 72.6
$t_{1/2}$ (h)	(n = 26)	(n = 26)	(n = 26)
Median (range)	22.4 (13.2–45.8)	23.2 (15.4–77.1)	25.2 (13.7–39.7)
Mean ± SD	25.1 ± 8.7	25.4 ± 11.9	24.7 ± 6.9
CL/F (l h^{-1})	(n = 26)	(n = 26)	(n = 26)
Geometric mean (range)	149.5 (40.8–349.5)	148.7 (41.5–323.6)	158.7 (51.1–393.7)
CV (%)	47.4	44.3	46.5
Arithmetic mean ± SD	168.1 ± 79.6	164.7 ± 73.0	178.3 ± 83.0

*MP29-02 formulation without FLU; †Meda Pharmaceuticals.

solubility (~0.1 µg ml^{-1}) and high first pass metabolism of the swallowed fraction of dose [14]. Previous bioavailability studies of FP nasal spray products were hampered by limitations in bioanalytical assay sensitivity, which required repeated administrations of supra-therapeutic doses [13, 14]. Administration with high doses of up to 800 µg required larger dose volumes that could exceed amounts readily retained by the nasal mucosa and escape nasal absorption [13, 14]. Therefore, these historical studies with large doses may not accurately reflect the true nasal deposition and absorption that would be associated with the use of therapeutic doses.

For the quantification of FP, we employed a highly sensitive LC-MS/MS with a LLOQ of 0.25 pg ml^{-1} that was approximately 80-fold more sensitive than bioanalytical assays used previously (e.g. 20 pg ml^{-1}) [16]. Hence, the studies could be conducted with doses reflecting the approved maximum single doses for AZE and FP and at

Table 4

Study 1: Statistical analysis of primary PK outcomes: Fluticasone propionate C_{max} and AUC(0,t_{last}) point estimates (PE) and 90% CIs for MP29-02 : MP29-02-FP-mono and MP29-02 : FP-BI

	AUC(0,t_{last}) (PP)			C_{max} (PP)		
		90% CI			90% CI	
	PE (%)	Lower limit (%)	Upper limit (%)	PE (%)	Lower limit (%)	Upper limit (%)
MP29-02 : MP29-02-FP-mono	93.55	83.60	104.68	91.01	82.53	100.37
MP29-02 : FP-BI	161.13	137.13	189.34	157.43	132.48	187.09

Table 5

Study 2: Statistical analysis of primary PK outcomes: Azelastine C_{max} and AUC(0,t_{last}) point estimates (PE) and 90% CIs for MP29-02 : MP29-02-AZE-mono and MP29-02 : Astelin®

	AUC(0,t_{last}) (PP)			C_{max} (PP)		
		90% CI			90% CI	
	PE (%)	Lower limit (%)	Upper limit (%)	PE (%)	Lower limit (%)	Upper limit (%)
MP29-02 : MP29-02-AZE-mono	98.82	90.96	107.37	102.67	92.12	114.44
MP29-02 : Astelin®	105.50	95.60	116.43	107.26	92.56	124.30

the same time the intended maximum daily dose of MP29-02. The assay was sufficiently sensitive to quantify systemic FP concentrations over 24 h in all but one of the study subjects and the sensitivity met current bioequivalence guideline requirements [17]. Interestingly, the FP serum concentration–time profiles displayed discrete double peak phenomena (Figure 1), possibly reflecting a biphasic nature of nasal FP absorption from the suspensions, i.e. a rapid absorption of the fraction of dose dissolved in the formulation and more sustained absorption from slowly dissolving suspended particles.

Plasma AZE profiles from the three treatments were nearly identical and the calculated primary PK parameters confirm that all products display similar *in vivo* performance in terms of AZE bioavailability (i.e. C_{max} and AUC, Tables 3 and 5, Figure 2). Results indicate that the FP component in MP29-02 does not affect the rate or extent of AZE absorption and does not appear to alter the subsequent *in vivo* disposition (i.e. distribution, metabolism and clearance) of AZE. In addition, the data confirm that the excipients included in the MP29-02 formulation do not impair AZE bioavailability compared with the marketed Astelin® nasal spray formulation. Taken together, neither formulation effects nor the presence of FP changed the overall AZE bioavailability and systemic exposure from MP29-02 as compared with the marketed product (Astelin®).

Serum FP profiles following MP29-02 and the MP29-02-based FP single entity products confirmed that both products displayed comparable *in vivo* performance (Tables 2 and 4, Figure 1), indicating negligible effect of AZE on FP bioavailability and disposition in MP29-02-based formulations. However, both MP29-02-based formulations showed a higher peak and total exposure compared with the FP marketed comparator product (FP-BI).

However, it should be noted that the systemic exposure of FP with MP29-02 nasal spray is still very low with mean peak concentrations of only 10–12 pg ml^{-1} or less. Estimates of absolute bioavailability based on an early i.v. FP study with a 250 µg i.v. dose [18] are consistent with low systemic FP bioavailabilities from the nasal spray products of only 1.86% for MP29-02 and 1.14% for FP-BI. It is unlikely that the difference in FP systemic exposure is clinically significant, especially considering the ~50% coefficient of variance associated with each AUC estimate for each of the products (Table 3).

Results from clinical safety trials in patients with AR support this conclusion. A study with FP nasal spray at doses eight times higher than the recommended daily FP dose revealed no effects on HPA-axis function, as evaluated by plasma cortisol response to a short cosyntropin test or 24 h urinary excretion of free cortisol [19–21].

Similarly, a study on the PK–PD relationship between the systemic exposure to FP and suppression of plasma cortisol secretion in healthy adult subjects indicates that the low systemic FP concentrations measured in the present investigation are not clinically meaningful [22]. The established PK–PD model is independent of dose and route of administration and showed that a total FP systemic exposure level (i.e. AUC) that is required to result in the half-maximum reduction in plasma cortisol concentrations (i.e. FP AUC_{50}) is about 3200 pg ml^{-1} h (95% CI 2.800, 3700 pg ml^{-1} h). The report indicates that the total FP exposure values which have been observed in the present study (about 60 pg ml^{-1} h) are generally very low and translate to systemic FP exposures that are about 45- to 60-fold below the exposure that would be required for a 50% suppression of cortisol secretion.

The PK–PD model also suggests that FP AUC values below 500 pg ml^{-1} h are unlikely to cause significant suppression of cortisol secretion, which represents a safety factor of about 8 for the susceptibility of HPA axis suppression.

The maximum approved daily dose for FP monoproducts in the United States and Europe is 400 µg day^{-1}. This is twice as high as the intended daily dose of MP29-02 (one puff per nostril twice daily = 200 µg day^{-1}). Therefore, with regular use of MP29-02 patients with AR will be exposed to less overall FP than with currently available FP single entity nasal sprays that are established as safe.

An explanation for the observed increase in FP bioavailability with MP29-02 could involve higher spray volume with MP29-02 (i.e. 137 *vs.* 100 µl) and difference in droplet size distribution (DSD, i.e. distribution of surface/volume of generated droplets). Further, the MP29-02 formulation has lower viscosity (41 cP) as compared with the marketed FP comparator product (70 cP). Together, the lower viscosity, the larger spray volume and the finer DSD profile of MP29-02 contributed to notable spray pattern improvements, including superior dispersion, larger spray pattern diameter and total area as compared with the FP-BI (data on file, MEDA Pharma, Bad Homburg, Germany). These biopharmaceutical characteristics of MP29-02 may result in improved nasal-mucosal distribution and a larger nasal-mucosal surface contact area for FP absorption. These properties may contribute to the improved clinical efficacy of MP29-02 as reported from a recent clinical trial in AR [23].

Competing Interests

Research funding for design and conduct of this study, collection, management, analysis and interpretation of the data was sponsored by MEDA Pharma GmbH & Co. KG, Germany.

UM, UP and JM are employees of MEDA Pharma GmbH & Co. KG.

HD, RH, and JB have been engaged by MEDA Pharma as expert advisors for various aspects of the studies. These authors received financial support for research and consulting services from MEDA Pharma. HM has been contracted by MEDA Pharma as bioanalytical expert advisor and service provider for the bioanalytics of fluticasone and has received financial support for these services.

None of these authors received payments for their contributions to the manuscript.

All authors contributed significantly to the analysis and interpretation of data, were involved in drafting the manuscript or revising it critically for important intellectual content and gave final approval of the version to be published.

The authors acknowledge Christine Kolb and Uwe Bürmann (MEDA Pharma) for their valuable contributions to the planning and management of the studies, Peter Romeis and Dr Dorothee Krone (prolytic GmbH, Frankfurt, Germany) for the bioanalytics of azelastine and conduct of pharmacokinetic analyses, Uwe Kraft for data management and the clinical team of ClinPharmCologne (Cologne, Germany) for the clinical conduct of the studies.

REFERENCES

1 Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, Zuberbier T, Baena-Cagnani CE, Canonica GW, van Weel C *et al*. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen. Allergy 2008; 63 (Suppl. 86): 8–160.

2 Brozek JL, Bousquet J, Baena-Cagnani CE, Bonini S, Canonica GW, Casale TB, van Wijk RG, Ohta K, Zuberbier T, Schünemann HJ. Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines: 2010 revision. J Allergy Clin Immunol 2010; 126: 466–76.

3 Wallace DV, Dykewicz MS, Bernstein DI, Blessing-Moore J, Cox L, Khan DA, Lang DM, Nicklas RA, Oppenheimer J, Portnoy JM, Randolph CC, Schuller D, Spector SL, Tilles SA, Joint Task Force on Practice, American Academy of Allergy, Asthma & Immunology, American College of Allergy, Asthma and Immunology, Joint Council of Allergy, Asthma and Immunology. The diagnosis and management of rhinitis: an updated practice parameter. J Allergy Clin Immunol 2008; 122 (Suppl.): S1–84.

4 LaForce C. Use of nasal steroids in managing allergic rhinitis. J Allergy Clin Immunol 1999; 103: S388–94.

5 Bousquet J, Bachert C, Canonica GW, Casale TB, Cruz AA, Lockey RJ, Zuberbier T. Unmet needs in severe chronic upper airway disease (SCUAD). J Allergy Clin Immunol 2009; 124: 428–33.

6 Ratner PH, Hampel F, Van Bavel J, Amar NJ, Daftary P, Wheeler W, Sacks H. Combination therapy with azelastine hydrochloride nasal spray and fluticasone propionate nasal spray in the treatment of patients with seasonal allergic rhinitis. Ann Allergy Asthma Immunol 2008; 100: 74–81.

7 Bachert C, Hampel F, Sacks H, Maus J, Munzel U, Price D, Bousquet J. MP29-02 and time to response in the treatment of seasonal allergic rhinitis compared to marketed antihistamine and corticosteroid nasal sprays. Allergy 2011; 66 (Suppl. s94): 285.

8 Bachert C, Carr W, Sacks H, Munzel U, Maus J, Price D, Bousquet J. Time to response and onset of action of MP29-02 in the symptomatic treatment of seasonal allergic rhinitis. Allergy 2011; 66 (Suppl. s94): 919.

9 Carr W, Bernstein J, Sacks H, Price D, Bachert C, Bousquet J. MP29-02 in the symptomatic treatment of seasonal allergic rhinitis – a comparative fall study. Allergy 2011; 66 (Suppl. s94): 920.

10 Meltzer E, Carr W, Sacks H, Price D, Bachert C, Bousquet J. MP29-02 in the symptomatic treatment of seasonal allergic rhinitis – a comparative spring study. Allergy 2011; 66 (Suppl. s94): 286.

11 European Medicines Agency, Committee for Medicinal Products for Human Use (CHMP). Guideline on Clinical Development of Fixed Combination Medicinal Products. CHMP/EWP/240/95 Rev. 1; 19 February 2009.

12 Hermann R, Locher M, Siebert-Weigel M, LaVallee N, Derendorf H, Hochhaus G. Intranasal loteprednol etabonate in healthy male subjects: pharmacokinetics and effects on endogenous cortisol. J Clin Pharmacol 2004; 44: 510–9.

13 Daley-Yates PT, Baker RC. Systemic bioavailability of fluticasone propionate administered as nasal drops and aqueous nasal spray formulations. Br J Clin Pharmacol 2001; 51: 103–5.

14 Daley-Yates PT, Kunka RL, Yin Y, Andrews SM, Callejas S, Ng C. Bioavailability of fluticasone propionate and mometasone furoate aqueous nasal sprays. Eur J Clin Pharmacol 2004; 60: 265–8.

15 Riethmueller-Winzen H, Peter G, Buker KM, Romeis P, Borbe HO. Tolerability, pharmacokinetics and dose linearity of azelastine hydrochloride in healthy subjects. Arzneimittel-Forschung/Drug Research 1994; 44: 1136–40.

16 Callejas S, Biddlecombe R, Jones A, Joyce K, Pereira A, Pleasance S. Determination of the glucocorticoid fluticasone propionate in plasma by automated solid-phase extraction and liquid chromatography-tandem mass spectrometry. J Chromatogr 1998; 718: 243–50.

17 European Medicines Agency, Committee for Medicinal Products for Human Use (CHMP). Guideline on the Investigation of Bioequivalence. CPMP/QWP/EWP/1401/98 Rev. 1; 20 January 2010.

18 Mackie AE, Ventresca GP, Fuller RW, Bye A. Pharmacokinetics of intravenous fluticasone propionate in healthy subjects. Br J Clin Pharmacol 1996; 41: 539–42.

19 Meltzer EO, Orgel HA, Bronsky EH, Furukawa CT, Grossman J, LaForce CF, Lemanske RF Jr, Paull BD, Pearlman DS, Ratner PH, Spector SL, Tinkelman DG, van As A, Rogenes PR. A dose-ranging study of fluticasone propionate aqueous nasal spray for seasonal allergic rhinitis assessed by symptoms, rhinomanometry, and nasal cytology. J Allergy Clin Immunol 1990; 86: 221–30.

20 Van As A, Bronsky E, Grossman J, Meltzer E, Ratner P, Reed C. Dose tolerance study of fluticasone propionate aqueous nasal spray in patients with seasonal allergic rhinitis. Ann Allergy 1991; 67: 156–62.

21 Nathan RA, Bronsky EA, Fireman P, Grossman J, LaForce CF, Lemanske RF Jr, Pearlman DS, Ratner PH, Rogenes PR. Once daily fluticasone propionate aqueous nasal spray is an effective treatment for seasonal allergic rhinitis. Ann Allergy 1991; 67: 332–8.

22 Mackie AE, Bye A. The relationship between systemic exposure to fluticasone propionate and cortisol reduction in healthy male volunteers. Clin Pharmacokinet 2000; 39 (Suppl. 1): 47–54.

23 Hampel FC, Ratner PH, Van Bavel J, Amar NJ, Daftary P, Wheeler W, Sacks H. Double-blind, placebo-controlled study of azelastine and fluticasone in a single nasal spray delivery device. Ann Allergy Asthma Immunol 2010; 105: 168–73.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1
Composition of the study medication
Table S2
Subjects excluded from PP population: (a) Study 1 (FP) and (b) Study 2 (AZE)
Table S3
Sensitivity analysis for study 1 (FP) – Descriptive statistics of PK parameters
Table S4
Sensitivity analysis for study 1 (FP) – Results from crossover ANOVA
Item S1
FP serum samples
Item S2
LC-MS/MS assay for FP
Item S3
AZE plasma samples
Item S4
LC-MS/MS assay for AZE
Item S5
Pharmacokinetic and statistical analyses
Item S6
Sensitivity analysis for study 1 (FP)
Item S7
Safety data

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.